

**Material and methods:** The eligibility criteria included either histologically or cytologically documented stage IIIB or IV NSCLC, a performance status 0 or 1, an age of  $\leq 74$  years, adequate organ function, no prior chemotherapy, and written informed consent. S-1 was administered orally at a dose of 40 mg/m<sup>2</sup> bid (80 mg/m<sup>2</sup>/day) on days 1–14 of a 21-day cycle. CDDP was administered intravenously on day 1 of each cycle (level 0, 1, 2: 60, 70, 80 mg/m<sup>2</sup>). Treatment was started at level 1 (70 mg/m<sup>2</sup>), and the dose was increased to level 2 (80 mg/m<sup>2</sup>) on the basis of DLT. However, if severe toxicity or other safety-related problems occurred, the dose was reduced to level 0 (60 mg/m<sup>2</sup>). The MTD and RD were decided by globally evaluating toxicity, completion of treatment according to protocol, and response.

**Results:** Eighteen patients were enrolled (12 men; median PS = 1). One patient had DLT at level 1 (S-1/CDDP at 80/70). No DLT occurred at level 2 (S-1/CDDP at 80/80). However, some adverse events meeting the criteria for DLT occurred after 2 cycles, and 6 additional patients were given level 0 (S-1/CDDP at 80/60). No DLT occurred at level 0. On the basis of these results, level 1 was determined to be the MTD, and 60 mg/m<sup>2</sup> the RD of CDDP.

**Conclusions:** Level 0 (S-1/CDDP at 80/60) is the RD for S-1 plus CDDP in patients with advanced NSCLC. This is an active combination against advanced NSCLC, and a multicenter phase II study is now in progress (supported by Taiho Pharma Japan).

1477

POSTER

#### Jasmonates can overcome drug resistance induced by p53 mutations and P-glycoprotein expression

E. Flescher<sup>1</sup>, D. Reischer<sup>1</sup>, O. Fingrut<sup>2</sup>, A. Harel<sup>2</sup>, I. Zan-Bar<sup>1</sup>. <sup>1</sup>Tel Aviv University, Human Microbiology, Tel Aviv, Israel; <sup>2</sup>Sepal Pharma, Ness-Zionna, Israel

**Background:** Jasmonates are plant stress hormones which we discovered as novel anti-cancer agents *in vitro* and *in vivo*. Given the urgent need for new chemotherapeutic agents to combat drug-resistant tumors, we decided to evaluate the activity of jasmonates against various types of drug-resistant cancer cells. We chose to study two major mechanisms of drug resistance: p53 mutations, occurring in more than half of human cancers, and multi-drug resistance mediated by P-glycoprotein (P-gp) expression.

**Methods:** Various drug resistant cells were studied. In addition, we compared the response of two pairs of clones: 1. B lymphoma cells – a clone expressing wild type p53 (29M6.2, wt p53) versus a clone expressing mutated p53 (29M6.10, mut p53); 2. Melanoma cells (B16-F10.9) – a clone expressing low levels of p-gp (low P-gp) versus a clone expressing high levels of P-gp (high P-gp). p53 and P-gp expression were determined by Western blotting analysis. Cytotoxicity was measured by a tetrazolium-based assay, and necrotic versus apoptotic death was determined by flow cytometry.

**Results:** Methyl jasmonate (MJ) killed efficiently prostate adenocarcinoma, glioblastoma and histiocytic lymphoma cells expressing mutated p53, as well as breast adenocarcinoma cells expressing both mutated p53 and high levels of P-gp. Jasmonic acid (JA) and MJ were each equally cytotoxic towards the wt p53 and mut p53 clones, whereas the mut p53 clone was resistant to treatment with the radiomimetic agent neocarzinostatin, as well as to bleomycin, and cisplatin. Neocarzinostatin and bleomycin induced an elevation in the p53 levels in the wt p53 clone whereas MJ did not. MJ induced mostly necrotic death (and apoptotic death in about 20% of the cells) in the wt p53 clone, but only necrotic death in the mut p53 clone. JA and MJ were each equally cytotoxic to the low P-gp and high P-gp clones, whereas the high P-gp clone was resistant to treatment with colchicine, doxorubicin and vinblastine. Inhibition of P-gp in the resistant cells, with the P-gp inhibitor PSC-833, sensitized the cells to the chemotherapeutic agents but did not increase the cytotoxicity of MJ.

**Conclusions:** Our findings suggest that jasmonates can circumvent the resistance of mutant p53- and P-gp-expressing cells towards chemotherapy. Jasmonates can induce p53-independent cell death, and do not appear to be P-gp substrates. Jasmonates are therefore promising candidates for the treatment of drug-resistant tumors.

1478

POSTER

#### Dual inhibition of mTOR and of the Insulin like Growth Factor Receptor-1 completely blocks the PI3K/Akt/mTOR pathway and results in a supra-additive anti-tumor effect: a mechanistic-based combination approach

S. Di Cosimo<sup>1</sup>, M. Scaltriti<sup>1</sup>, F. Rojo<sup>1</sup>, D. Val<sup>1</sup>, M. Guzman<sup>1</sup>, J. Seoane<sup>1</sup>, J. Jimenez<sup>1</sup>, J. Arribas<sup>1</sup>, F. Cognetti<sup>2</sup>, J. Baselga<sup>1</sup>. <sup>1</sup>Vall d'Hebron University Hospital, Medical Oncology, Barcelona, Spain; <sup>2</sup>Regina Elena Cancer Institute, Medical Oncology "A", Rome, Italy

RAD001, an oral derivative of rapamycin, inhibits mTOR, a protein kinase downstream of PI3K and Akt, involved in the regulation of cell growth,

proliferation and survival. We and others have previously shown that RAD001 has anti-tumor activity *in vitro* and *in vivo*. In our preclinical studies as well as in ongoing phase I pharmacodynamic study, RAD001 administration results in an increase in activated (phosphorylated) Akt (p-Akt). In prior studies (Di Cosimo, AACR 2005) we reported that gefitinib, an anti-epidermal growth factor receptor TKI, has the capacity to inhibit pAKT when given alone but does not fully reverse RAD001-induced pAkt suggesting that Akt activation as result of mTOR inhibition occurs via an alternative mechanism/s.

In exploratory studies, we observed a RAD001 dose-dependent induction of insulin receptor substrate-1 (IRS-1), which serves as a docking protein for SH2 domain containing proteins, including PI3K, both in prostate (DU145) and breast (MCF-7) cancer cell lines. The RAD001-induced increased expression of p-Akt and IRS-1 was also seen *in vivo* by immunohistochemical analysis of MDA-MB-468 human breast cancer xenografts. Since IRS-1 is tightly regulated by insulin-like growth factor receptor 1 (IGF-1R), we analyzed IGF-1R signaling in RAD001 treated cells. First, in DU145 cells, treatment with IGF-1 resulted in a strong induction and posterior degradation of IRS-1. Similarly, p-Akt was rapidly induced (30 minutes) and then gradually decreased (12 hours). Conversely, the addition of RAD001 resulted in a sustained induction of both IRS-1 and p-Akt, for up to 36 hours. In order to dissect the mTOR-dependent pathways that regulate Akt activation, experiments with siRNA demonstrated that a 70% knock down of the down-stream effector of mTOR S6K mimics the effect of RAD001 by increasing the levels of p-Akt and preventing the degradation of IRS-1. These results suggest that the inhibition of S6K by RAD001 results in a sustained activation of IGF-1R signaling which in turn results in the activation of Akt. Next, we demonstrated that the IGF-1R TKI NVP-AEW-541, at a dose of  $\geq 1 \mu\text{M}$  inhibits the IGF-1R pathway and prevents the activation of Akt induced by either siRNA-S6K or RAD001. Combined treatment with RAD001 (3, 6 and 12 nM) and NVP-AEW-541 (1.5, 3 and 6  $\mu\text{M}$ ) resulted in supra-additive growth inhibitory effects in a panel of different cancer cell lines (DU145, BT-474, MDA-MB-468), as demonstrated by the median effect analysis of growth curve.

In summary, the IGF-1R pathway is involved in the activation of Akt by RAD001 and dual mTOR and IGF-1R targeting is a mechanistic-based combined anti-tumor strategy. Our findings provide a rationale for combining the inhibitors of mTOR with agents targeting growth factor receptors as a novel anticancer approach.

1479

POSTER

#### ZK-EPO, a novel epothilone B derivative, significantly inhibits tumor growth in a wide range of human tumor models

U. Klar<sup>1</sup>, B. Buchmann<sup>1</sup>, W. Schwede<sup>1</sup>, W. Skuballa<sup>1</sup>, R. Lichtner<sup>2</sup>, J. Hoffmann<sup>1</sup>. <sup>1</sup>Schering AG, Berlin, Germany; <sup>2</sup>Signature-Diagnostics, Potsdam, Germany

**Background:** Epothilones represent a novel class of natural products that stabilize microtubules and exhibit marked antiproliferative effects. ZK-EPO is a novel epothilone analog that has shown marked preclinical activity.

**Material and methods:** A broad panel of cell lines derived from normal cells or a variety of human tumors (eg, colorectal, prostate, breast) were exposed *in vitro* to different concentrations of ZK-EPO and compared with standard cytotoxic compounds (eg, paclitaxel, adriamycin, cisplatin, and camptothecin). *In vivo*, the efficacy of ZK-EPO was compared with standard cytotoxic compounds (eg, paclitaxel, cisplatin, doxorubicin) in human tumor xenografts. These included breast and pancreatic cancer, melanoma, ovarian (OVAR Ca-2, OVCAR 8, A2780/AD10), cervical (A431), and non-small-cell lung (NCI-H460) carcinomas that were grown in nude or SCID mice.

**Results:** *In vitro*, ZK-EPO showed significantly greater activity in all human tumor cell lines examined compared with paclitaxel, adriamycin, cisplatin or camptothecin, with a mean IC<sub>50</sub> value of  $< 1 \text{ nM}$  after 3 days' incubation. ZK-EPO also exhibited significant activity compared with paclitaxel, and all other agents examined, in cell lines expressing marked resistance to the standard cytotoxics. ZK-EPO showed no toxicity against quiescent non-malignant cells (HUVEC, HaCat) and, unlike paclitaxel, showed high accumulation in tumor cell nuclei *in vitro*. *In vivo*, ZK-EPO was highly effective in a wide spectrum of human tumor models, both non-resistant (OVCAR 8, OVCAR 3, MaTu, OVAR Ca-2) and resistant (MaTu/Dox, NCI/ADR, A2780/AD10), exhibiting superior activity compared with paclitaxel. It was also active in melanoma and pancreatic cancer models representing tumors intrinsically resistant to almost every chemotherapeutic agent.

**Conclusions:** ZK-EPO exhibits significantly better activity compared with paclitaxel, and other agents, in a range of *in vitro* and *in vivo* tumor models. ZK-EPO is the first fully synthetic epothilone in clinical trials designed to overcome multi-drug resistance and to combine high efficacy with an improved therapeutic window. ZK-EPO's ability to circumvent resistance mechanisms in preclinical models suggests that it may postpone or prevent the development of resistance when used in the clinic.